Judul Disertasi

# KAJIAN SENYAWA BERPOTENSI ANTIANKER DAUN *POLYALTHIA GLAUCA* (Hassk.) BOERL: IDENTIFIKASI SENYAWA SITOTOKSIK TERHADAP SIKLUS SEL DAN APOPTOSIS SEL KANKER SERVIKS (Hela) IN VITRO

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Genus Polyalthia familia Annonaceae mempunyai peran penting dalam pengobatan karena mengandung senyawa aktif yang terbukti memiliki berbagai aktivitas, antara lain sitotoksik pada berbagai sel kanker, antiinflamasi, sebagai analgesik, anti-HIV, antibakteri, serta antioksidan. *Polyalthia glauca* (Hassk.) Boerl. merupakan anggota genus yang belum banyak dikaji kandungan senyawa aktif serta bioaktivitasnya. Menimbang potensi khasiat obat terutama antikanker serta belum ada informasi mengenai senyawa yang memiliki aktivitas antikanker serviks pada spesies tersebut maka kajian senyawa antikanker pada spesies ini dilakukan. Tujuan penelitian ini adalah mengisolasi dan mengidentifikasi senyawa sitotoksik daun *P. glauca,* mengkaji potensi sitotoksik, efek senyawa tersebut terhadap siklus sel dan apoptosis, serta pengaruh senyawa terhadap ekspresi protein p53, pRb, Bcl-2, caspase-9, dan caspase-3 sel HeLa *in vitro.*

Isolasi senyawa bioaktif dilakukan dengan metode *bioassay guided fractionation*, monitoring dilakukan dengan KLT dan *MTT assay* terhadap sel HeLa. Ekstraksi serbuk kering daun dilakukan dengan maserasi bertingkat, berturut-turut dengan kloroform, metanol dan air. Ekstrak paling aktif yaitu ekstrak kloroform daun (EKDP) difraksinasi dengan *vacuum liquid chromatography* (VLC) dengan peningkatan polaritas eluen (n-heksan:etil asetat,v/v), dihasilkan 16 fraksi, fraksi dengan profil KLT yang mirip digabung sehingga diperoleh 6 fraksi/fraksi gabungan. Selanjutnya dilakukan pemisahan senyawa dari fraksi paling aktif yaitu fraksi III yang dielusi dengan n-heksan : etil asetat (6:4;v/v) dengan kromatografi lapis tipis preparatif dan diperoleh 3 senyawa. Senyawa yang didapatkan diuji kemurniannya secara KLT, selanjutnya dilakukan identifikasi struktur senyawa aktif dengan spektroskopi UV, IR, 1HNMR,13CNMR,DEPT 1350, COSY, HMQC, HMBC, dan Massa. Uji sitotoksisitas senyawa aktif dilakukan terhadap sel HeLa dengan metoda *MTT assay.* Pengaruh senyawa aktif terhadap siklus sel HeLa dianalisis dengan *Flow cytometry*. Kajian induksi apoptosis dianalisis dengan pengamatan fragmentasi DNA sel HeLa. Ekspresi protein p53, pRb, caspase-9, caspase-3, dan Bcl-2 dideteksi dengan teknik imunositokimia, kemudian dianalisis secara semikuantitatif menggunakan *Allred scoring*.

Hasil penelitian menunjukkan bahwa daun *P. glauca* mengandung senyawa sitotoksik terhadap sel HeLa *in vitro*. Senyawa sitotoksik yang diperoleh termasuk dalam golongan secolignan yaitu Glaucinitinitin A *: 4-[(3,4-Dimethoxy-phenyl)-(3,4,5-trimethoxy-phenyl)-methyl]-3-methylene-dihydro-furan-2-one*, Glaucinitinitin B *: 4-[(3,4-Dimethoxy-phenyl)-(3,4,5-trimethoxy-phenyl)-methyl]-3-methylene-dihydro-furan-2-one,* dan Glaucinitinitin C: *4-[Bis-(3,4,5-trimethoxy-phenyl)-methyl]-3-methylene-dihydro-furan-one*. Hasil uji sitotoksisitas ketiga senyawa terhadap sel HeLa masing-masing IC50 berturut-turut adalah 12,11±0,29 µg/mL, 6,62±0,2 µg/mL, dan 9,02±0,2µg/mL. Hasil uji ketiga senyawa terhadap distribusi sel HeLa pada

siklus sel menunjukkan bahwa senyawa aktif 1 mampu menghambat siklus sel pada fase G0-G1 sebesar 70,65 %, sedang senyawa aktif 2 dan 3 mampu meningkatkan jumlah sel pada fase G2-M sebear 19,03 dan 19,1%.

Ketiga senyawa juga terbukti mampu menginduksi apoptosis sel HeLa serta mampu menginduksi ekspresi p53, pRb, caspase-9, caspase-3, dan mampu menekan ekspresi gen antiapoptosis Bcl-2 sel HeLa. Dari hasil penelitian dapat disimpulkan bahwa senyawa sitotoksik daun *P. glauca* merupakan senyawa baru golongan secolignan yaitu Glaucinitin A, Glaucinitin B, dan Glaucinitin C dan ketiga senyawa berpotensi memodulasi siklus sel serta mampu menginduksi apoptosis sel kanker serviks He La. **[Kata kunci: *Polyalthia glauca,* sitotoksik, sel HeLa, siklus sel, apoptosis.]**

**STUDY OF ANTICANCER COMPOUNDS OF POLYALTHIA GLAUCA (HASSK)BOERI LEAVES: ISOLATION AND IDENTIFICATION OF CYTOTOXIC COMPOUNDS, MECHANISM ON HELA CELL CYCLE AND APOPTOSIS.** Polyalthia (Annonaceae) is considered to be a medicinal importance because of the presence of clerodane diterpenoids and alkaloids in various parts of the plant. They have many bioactivities such as cytotoxicity on many variate of cancer cell line, antiinflamation, analgesic, anti-HIV, antibacteria, and antioksidan. Compound as an anticancer activity in *P. glauca* has not been reported. Therefore the objective of this research were to isolate and identify the structure of cytotoxic compound, to study the cytotoxic activity of it on the HeLa cell line, to study the effect of it on cell cycle and apoptotic induction, and to investigate the influence it on p53, pRb, Bcl-2, caspase-9 and caspase-3 expression on HeLa cell line *in vitro* of *P. glauca* leaves.

The bioactive compound isolation was done by bioassay guided fractionation. Monitoring compound was done by TLC and cytotoxic effect by MTT assay. The dried powder leaves of *P. glauca* was extracted by maserated using chloroform, methanol and water.The active extract was fractionated by vacuum liquid column chromatography using 16 eluents that increased in polarity (n-hexane: ethyl acetate, v/v). The fractionation produced 16 fractions , and the fractions that had similar pattern on KLT were combined and obtaining 6 combined fractions. MTT assay results showed that the most active fraction (FIII) was the one that was eluted by n-heksan : ethyl acetat (6:4;v/v) This fraction was further separation by preparative thin layer chromatography and obtained 3 compounds. Compounds was tested for purity by TLC, further active compound structures were elucidated by UV, IR, NMR (1D and 2D) and Mass Spectroscopy. Active compounds were evaluated for their cytotoxic activities on HeLa cell lines by MTT assay method. Effect of active compounds on HeLa cell cycle were analyzed by flow cytometry. Apoptosis induction on HeLa cell line by the active compounds were analyzed by observation of DNA fragmentation. Expression of p53, pRb, Bcl-2, caspase-9 and caspase-3 were detected by immunocytochemistry technique, then analyzed semiquantitatively using the Allred scoring.

The results showed that cytotoxic compound in *P. glauca* leaves belonging to the group of secolignan, the active compound 1 as Glaucinitinitin A was 4 - [(3,4-Dimethoxy-phenyl) - (3,4,5-trimethoxy-phenyl) -methyl] -3-methylene-dihydro-furan-2 -one, the active compound 2 as Glaucinitinitin B was 4 - [(3,4-Dimethoxy-phenyl) - (3,4,5-trimethoxy-phenyl) -methyl] -3-methylene-dihydro-furan-2-one, and the active compound 3 as Glaucinitinitin C was 4- [Bis- (3,4,5-trimethoxy-phenyl) -methyl] -3-methylene-dihydro-furan-one, and cytotoxicity assay of those three compounds on HeLa cells showed $IC_{50}$ was 12,11±0,29 µg/mL, 6,62±0,2 µg/mL, 9,02±0,2µg/mL respectively. Results on the molecular assay showed that the Glaucinitin A was able to inhibit the cell cycle at G0-G1 phase amounted to 70.65%, while the active Glaucinitin B and Glaucinitin C affect at the G2-M

phase amounted to 19,03 and 19,1 %. The active compounds also proved to be able to induce apoptotic on HeLa cells and capable of inducing the expression of p53, pRb, caspase-9, caspase-3, and could suppress the expression of Bcl-2 HeLa cells.
From the research can be concluded that three new cytotoxic compound of *P. glauca* leaves have been isolated which potential to modulate cell cycle and capable to induce apoptosis in cervical cancer cells HeLa. [**Key words : *Polyalthia glauca,* cytotoxicity, HeLa cell line, cell cycle, apoptosis**]